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END RESULTS OF DESICCATION AND RESPIRATION IN SUCCULENT PLANTS

D. T. MACDOUGAL, E. R. LONG AND J. G. BROWN

ABSTRACT[1]

A large number of seed-plants absorb a much greater quantity of water during certain seasons or in a certain stage of their development than is lost, and the surplus may accumulate in exaggerated cortical or medullary tracts in the roots, stems or leaves. The stored water in many instances holds soluble carbohydrates, or these substances may be present in the form of starch, etc. Large water balances are especially characteristic of the succulents of semi-arid regions in which the rainfall comes within well-defined annual periods. This feature is especially illustrated by the cacti, certain forms of which were used for experimental material in the studies described in this paper.

A number of large, sound individuals of Echinocactus, and of severed joints of flat stems of Opuntia were deprived of a water supply, and compelled to carry on existence at the expense of accumulated water and food-material. Some of the preparations were exposed to the full illumination to which they were accustomed, and others were placed in diffuse light, obtaining differential effects in water-loss, respiration, disintegration of acids, and photosynthesis. The principal generalizations arising from the studies are as follows:

1. Echinocactus in diffuse light may lose as much as one two-thousandth part of its weight in one day, immediately following the excision of its root-system. The same plant six years later, under equivalent conditions except that its weight had been reduced nearly a third, lost no more than one part in seventeen thousand of its weight in one day.

2. An Echinocactus weighing 38 kg., of which 90–95 per cent may be estimated as water, lost 3.5 kg., or one-tenth of its total water, in the first year of isolation in diffuse light. In the sixth year the loss was one-twentieth of the water supply at the beginning of that year.

3. Echinocacti in the open lost 38–45 per cent of their original weight during the period from June to November inclusive. Individuals in the diffuse light of the experimental rooms lost 7 or 8 per cent in the same period.

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[1] The manuscript of this paper was received June 12, 1915. This abstract was preprinted, without change, from these types and was issued as Physiological Researches, Preliminary Abstracts, vol. 1, no. 6, August, 1915.

4. An Echinocactus in the open may survive no more than two years at the expense of its surplus food-material and water. Similar plants in diffuse light have been seen to be sound after six years of starvation, although the effects were marked.

5. Prolonged confinement in diffuse light results in a decrease in density of sap in Echinocactus. Exposure in the open, with consequent rapid loss in weight, may be followed by an increase or by a decrease in the density of the sap.

6. Decrease in the density of sap is to be attributed to a disintegration of the carbohydrates, which, in Echinocactus No. 7, amounted to 13 per cent of the dry weight of the cortex. The destruction of material was extended to include the walls of whole masses of tissue in the cortex.

7. Increase in the density of the sap might result from rapid evaporation, which altered the proportions of water and dissolved substances, or by the addition of photosynthetic products.

8. The proportion of reducing sugars is greatest in the peripheral tissues of normal plants, in connection with the photosynthetic activity localized here, and decreases through the cortex to the central cylinder. The reduction which takes place in desiccation and starvation reverses the distribution of these substances, the greatest proportion after desiccation being found in the inner cortex and the total amount being reduced.

9. Non-reducing soluble sugars, which are present in only minute proportions if at all in normal Echinocacti, are noticeable constituents of the sap of desiccated plants.

10. The acidity of the tissues is due to certain modifiable features of respiration. Acidity of plants which have undergone long continued desiccation and starvation is low, since the amount of carbohydrate from which they are derived has been decreased.

11. Catabolism in extended desiccation and starvation eventually breaks down the plasmatic colloids, and includes hydrolysis of the cell-walls of the cortex.

12. The water-absorbing capacity of cortical tissues of Echinocactus is a resultant of the osmotic activity of the solutions and of the hydratation power of the colloids, consequently great diversity was found in the various specimens examined with regard to this feature.

13. Echinocactus is capable of growth in the apical region, in plants in which water loss and disintegration of the carbohydrates (including hydrolysis of the cortical walls) has reached an advanced stage.

14. The rate of loss in weight of an Echinocactus, largely due to evaporation, is not correlated with the degree of succulence (proportion of amount of water present to superficial area of body), or with the density of the sap, but is to be attributed to morphological causes.

15. The loss in weight of Opuntia in full sunlight and in diffuse light is

not very different during the first thirty-five days of exposure, and is practically the same after that length of time. The position of the flattened joints in the open may modify the rate of loss.

16. Opuntia desiccating in the open shows an increase in dry weight, but a decrease in hydrolyzable carbohydrate, while the acidity is not markedly different from the normal, though slightly less. Desiccation in diffuse light results in increase of acidity, increase of dry weight (not as pronounced as in the open) and decrease in hydrolyzable carbohydrate.

17. The hydratation capacity of a joint is increased by desiccation in full sunlight, and is decreased by desiccation in diffuse light. The diminished capacity for absorption of water may be due to the high acidity in the latter case.

18. The difference in behavior of the two types of cacti in desiccation and starvation is correlated with definite physical features. Echinocactus has a globoid stem consisting largely of thin-walled cells, in which the accumulated food-material is in the form of soluble carbohydrates. Solid material and accessory colloids are noticeably lacking. The flattened joint of Opuntia is composed of a net-work of fibrovascular tissue. The fundamental tissue is rich in slime or mucilage, and somewhat higher in total hydrolyzable carbohydrates than is the fibrovascular tissue. The loss of water from the large, globose stems of Echinocactus is much more affected by illumination than in the flattened stems of Opuntia. The course of respiration in the thin stems of Opuntia is such that acids formed during the process are present in greater proportion, and vary more widely through the day, than in the large Echinocacti. Some connection with the hydratation of the slimes or mucilages is suggested.

19. Isolated individuals of succulent species survive varying periods when separated from a moist substratum. If the conditions for photosynthesis are inadequate death may ensue from starvation. The disintegration of solid material in diffuse light may be such that the proportion of water in the tissues may be but little changed after several years of depletion.

Loss in weight in full illumination may not greatly exceed 50 per cent of the water present, without producing death by desiccation. The specific action of such excessive loss of water has not been determined.

20. Extended desiccation and starvation made no alteration in the integument of Echinocactus, but in a plant which had been thus treated for seventy-three months the cuticle was thicker than normal while the outer walls of the epidermal cells were thinner. Cytoplasm and nuclei in the epidermal system were reduced, but new cork layers were being formed as in normal plants. Cell division was seen in the epidermal layer at the bottom of the grooves of the stem. The stomata remained permanently open and many were in a collapsed condition. Guard cells of stomata differed from the normal in having the anterior walls thinner as compared with the posterior walls.

21. The palisade layer was thinner in desiccated than in normal plants of Echinocactus. The cytoplasm was reduced to small masses in the angles of the cells, and the nuclei were variously deformed and reduced in size. Vacuoles had disappeared from the nucleoplasm and a thickened granular layer was present in the peripheral portion.

22. The most pronounced effects of desiccation and starvation were exhibited by the cortex of Echinocactus. The changes noted as having been seen in the palisade tissue were followed by the entire disappearance of the protoplasts and the hydrolysis of the cell walls. The consequent disintegration of cell masses formed lacunae as large as 8 cc.

23. Some of the effects of desiccation and starvation were to be found in the medulla of Echinocactus plants undergoing extended desiccation and starvation, but to a lesser degree. Disintegration of cell walls was observed in restricted areas. No change appeared to be produced in the vascular bundles by desiccation and starvation.

24. Early stages of the changes noted above, such as the reduction in cytoplasm and nuclei of cells, deformation and peripheral thickening of nuclei, and hydrolysis of cell walls, were found in plants which had been desiccated in diffuse light for only ten months.

25. An Echinocactus which had been desiccated for forty-two months and then placed under normal conditions in the soil for twenty-two months, did not entirely regain the normal condition. The epidermal system was fairly normal, excepting irregularity in proportional thickness of anterior and posterior walls of stomatal guard cells. Nuclei of the palisade cells were below normal in size, and only one was seen that had regained normal shape. The peripheral, thickened, granular layer was still present in many cases. The cortex also retained irregularities of cell wall and nucleus, as effects of the starvation and desiccation. Recovery was most advanced in the outer part of the cortical region. Cell walls in the outer cortex varied from two to ten micra in thickness while in the inner cortex the variation was from less than one micron to over twenty micra. The inner cortex of this recuperating plant was characterized by some nuclei which were larger than the normal.

GENERAL COURSE OF DEPLETION IN STARVING SUCCULENTS

D. T. MACDOUGAL

The senior author began a series of tests to determine the rate, course and extent of the water-loss in massive succulents in 1908. Selected individuals of Echinocactus, Carnegiea and other plants with a relatively large water-balance growing in the Tucson region were taken from their habitats and placed upon stands which supported them at the height of a meter, in such manner that the light exposure was normal as to angle. Some were put in

this position in the open, exposed to the full force of the sun, and were subject to the high midsummer temperatures of the region. Others were placed in laboratory rooms in which the illumination was from ordinary side-windows, and the temperature was rarely altered by artificial heat. Its general course was more equable than that to which the plants in the open were subjected.

Survival in the open generally did not extend beyond two years as the depletion of the water-balance proceeded with such rapidity that more than half of the amount held by the normal plant in a turgid condition would be lost within that time. (See fig. 1.) No plant of any species tested survived a loss of 54 per cent of the original supply.

The course of desiccation under the conditions named has already been described in several papers.[2] Certain features of the variations in weight of the plants under observation, however, remained without adequate explanation. Among these is to be included the fact that the rate of water-loss decreases more rapidly than the ratio of succulence, which is the proportion of water present to the area of the transpiring surfaces. Thus the loss of 8 to 10 per cent of the water originally present in a succulent would be followed by a decrease of 50 per cent or more in the rate of loss. The concentration of the sap resulting from depletion of the water-balance increased the relative amount of ash, but did not increase the amount of acid present, a fact which was soon seen to depend upon the disintegrating action of light. The large, globoid stems of Echinocactus showed an increased proportion of total solids in the sap when desiccation ensued rapidly, as in the full blaze of sunlight, but when the process was allowed to continue into the second year the proportion of total solids decreased to about that of normally turgid plants. (See MacDougal [12], pages 76–78.)

Nearly all of the plants from which the above generalizations were derived were finally destroyed, or perished by starvation or drying. Among the survivors, however, was a large Echinocactus ("No. 7") which had been taken from the soil in November, 1908, and kept in a shaded room for more than six years. Partial records of this plant from 1908 to 1913 have been published. As this plant was taken for detailed examination in connection with the work of the present paper, however, it is necessary to recapitulate its history for the entire period during which it was kept under observation. This history is shown in table I. In continuation of the practice followed

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[2] MacDougal, D. T., and Spalding, E. S., The water-balance of succulents. Carnegie Inst. Wash., Pub. 141. 1910. MacDougal, D. T., The water-balance of desert plants. Ann. Bot. **26**: 71–93. 1912. NOTE.—Failure of the senior author to read proof, because of his absence in the Libyan desert, makes necessary the following corrections in this paper: Page 74, 5th line, read "40 grams," instead of "40 kg." Page 76, 2d line, read "4.248 kg." for "42.48 kg." Page, 77, 7th line from bottom, read "32 liters" for "32,000 liters."

———, The measurement of environic factors and their biologic effects. Pop. Sci. Monthly **84**: 417–433. 1914.

in the earlier papers, the weights here presented are those which will serve to show the course of loss during the winter and summer.

The winter and summer periods in the above table are arbitrarily limited by convenient dates of observation. Weights were obtained at irregular intervals, which might vary in length from a few days to a few weeks. A second arrangement of the data, to show variations during periods roughly

TABLE I

Seasonal variations in weight of Echinocactus No. 7

(Arranged to show loss during winter and summer periods)

DATE OF OBSERVATION	WEIGHT	LOSS SINCE LAST WEIGHING	AVERAGE DAILY RATE OF LOSS	REMARKS
	kilograms	*grams*	*grams*	
November 7, 1908	37.595			Taken from soil and placed on support
December 8, 1908	37.040	555	18	Cut surfaces not yet healed
March 23, 1909	36.120	920	9	Winter period
October 1, 1909	34.135	1985	10.3	First summer period
March 22, 1910	33.480	655	3.8	Second winter period.
October 3, 1910	32.055	1425	7.4	Second summer period
March 17, 1911	31.650	405	2.4	Third winter period
October 16, 1911	30.520	1130	5.3	Third summer period
May 16, 1912	30.120	400	1.9	Fourth winter period
September 25, 1912	29.110	1010	7.6	Fourth summer period: rate higher than 1911
March 20, 1913	28.800	310	1.7	Fifth winter period; rate lower than previous year
September 28, 1913	27.825	975	5	Fifth summer period; rate less than 1912
March 6, 1914	27.445	380	2.4	Sixth winter period; rate higher than previous period
October 4, 1914	26.495	950	5.2	Sixth summer period; rate higher than in previous summer
December 7, 1914	26.395	100	1.9	

corresponding to the years of the calendar, will therefore be useful in presenting other features of desiccation.

The greatest rate at which this plant lost weight was 18 g. daily during the first month after its removal from the soil, at which time the freshly cut surfaces of numerous roots and of other abrasions would facilitate loss of water. Chief interest, however, centers in the happenings after the tissues were normally enclosed. Examination of the records reveals the fact that during a short interval in the first summer the maximum rate was 11.6 g. daily. The seasonal average for the same summer was 10.5 g. daily, which

fell to 2.9 g. daily. A maximum of 5.5 g. daily, however, occurred in the sixth summer, a rate which was nearly as great as that of the previous summer (fig. 1).

The high maximum rate of water-loss from the plant after more than five years of desiccation may be associated with the fact that a large amount of new tissue was formed at the apex of the globose body during the period April to June, 1914. The rate of decrease in weight of the plant was seen to fall very rapidly, however (fig. 2), and in a manner not explainable by the

TABLE II

Annual variations in weight of Echinocactus No. 7

PERIOD	NO. OF DAYS	TOTAL LOSS	AVERAGE DAILY RATE OF LOSS
		kilograms	*grams*
November 7, 1908 to October 3, 1909	328	3.443	10.5
October 3, 1909 to October 3, 1910	367	2.086	5.4
October 3, 1910 to October 16, 1911	378	1.535	4
October 16, 1911 to September 25, 1912	345	1.430	4.4
September 25, 1912 to September 28, 1913	368	1.165	3.2
September 28, 1913 to December 7, 1914	435	1.280	2.9
Totals, 6 yrs. 1 mo.	2221	11.043	Average, 5

ratio of water present to the transpiring surface. In an earlier discussion of this matter it was said (MacDougal [12], page 90):

Five possible causes, which might have influenced the rate of transpiration of a desiccating succulent, present themselves. These are as follows: 1st, the increased concentration of the cell-sap, which was of such degree in the experiments as to increase osmotic pressures from 4 or 5 to 10 or 12 atmospheres, might retard evaporation from the cell-membranes; 2d, a diminution of the degree of succulence, or proportion of water per unit area of surface present might lessen evaporation; 3d, desiccation may result in alterations in the character of the outer membranes, or of any of the transpiring walls of the plant; 4th, desiccation may stimulate the formation of new tissues or the alteration of existing cells in such manner as to close openings through which water vapour might pass; and 5th, the positions of the surfaces might be shifted in such manner as to vary the exposure and lessen transpiration.

Livingston[3] has recently pointed out that a concentration of the sap even if carried to a point where an osmotic pressure of 100 atmospheres was exhibited, would not give a retardation of more than 10 per cent of the rate afforded by a pure water surface. It is evident, therefore, that this factor is negligible in the present discussion, as the increases in sap concentration that were found were not more than 5 or 6 atmospheres. The rate of loss

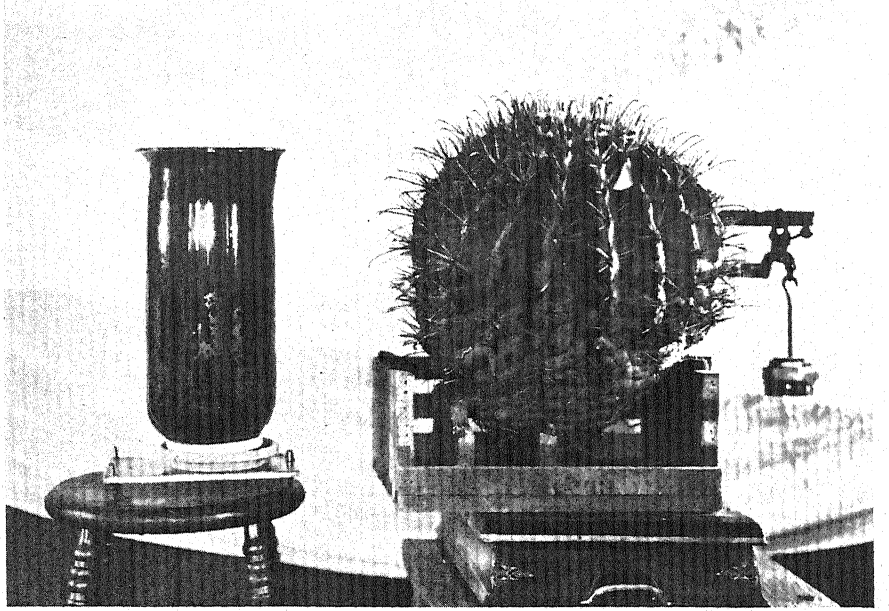

Fig. 1. Echinocactus wislizeni no. 7, December, 1915, after 73 months of desiccation and starvation. The cylinder at the left contains 9.5 kg. of water, the amount lost by the plant during the above period.

diminishes so much more rapidly than the estimated degree of succulence in slowly desiccating individuals, that it is impossible to escape the conclusion that other agencies are operative.

No further evidence bearing upon the matter was obtained, and the subject was allowed to rest until opportunity was obtained to make additional

[3] Livingston B. E., The relation of the osmotic pressure of the cell sap in plants to arid habitats. Plant World **14**: 153–164. 1911.

measurements. Early in 1914 it was decided to close the career of Echinocactus no. 7 after it had undergone its sixth summer of desiccation. Preparations were made for an anatomical and chemical examination of other individuals which had been subjected to periods of desiccation of varying length. The main inquiries concerned the possible alterations in the tissues and solutions of the cells, as a result of the desiccation, and the fate of the

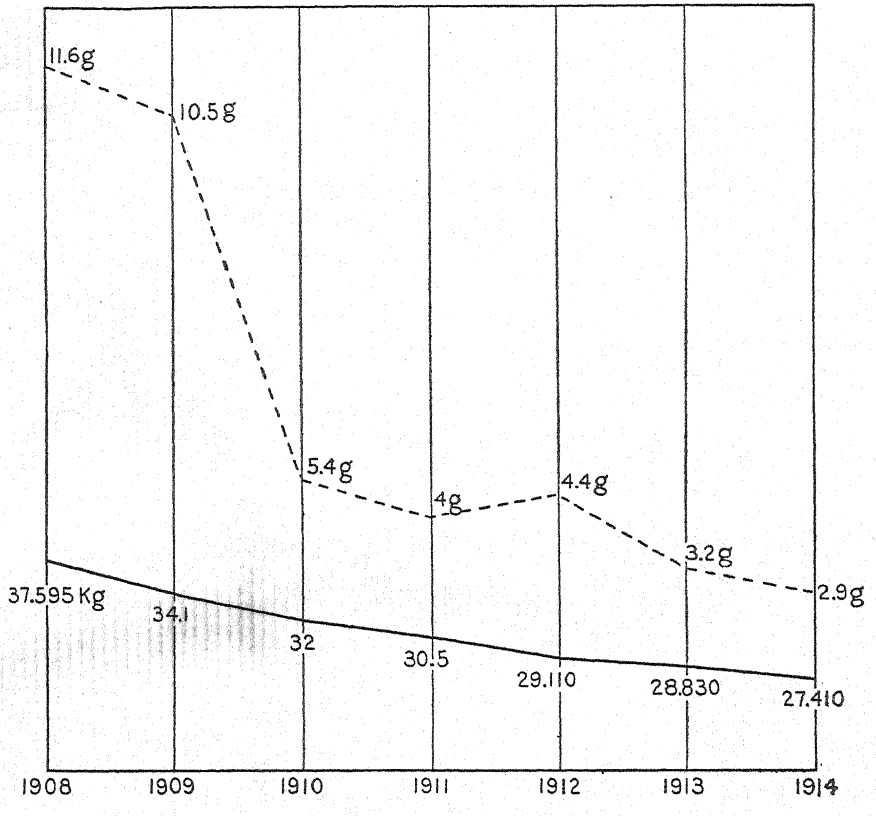

FIG. 2. The solid line shows the course of variation in weight of Echinocactus no. 7, from November, 1908, to December, 1914. The broken line, with its notations, illustrates the variations in rate of loss during this period.

surplus food-material and integral substances of the plasmatic colloids as a result of the continued respiration of the plants which were kept under "starvation" conditions.

The renewed attention given the matter was chiefly upon the basis of the results achieved by Dr. H. M. Richards and by Dr. H. A. Spoehr in their researches upon the variations in acidity and the general catabolic processes of succulents and other plants. It is to be noted that the starvation and

desiccation phenomena discussed are of an extent and duration not previously available to any physiologist. The details are given in the following sections of this paper.

CHEMICAL CHANGES ACCOMPANYING DESICCATION AND PARTIAL STARVATION

E. R. LONG

The attempt to obtain individuals which would illustrate the metabolic changes taking place at intermediate stages of desiccation, was begun in June, 1914, when six healthy plants of Echinocactus were taken up from the slopes west of the Desert Laboratory and placed on supports, three in the laboratory court exposed to full sunlight, and three within the laboratory in diffuse light, in a room with north exposure. The loss in weight was determined by weighing at intervals, as indicated in the following tables, and analyses were made after periods approximately of five, and of eight and one-half months of desiccation under the two sets of conditions. The rate of loss in weight varied little among the plants kept in diffuse light and not a great deal among those exposed to the full sunlight outdoors. Curves of variations of one in the open and one indoors are given in figure 2. The complete record of water-loss for all individuals is given in table III.

TABLE III

Loss in weight of desiccating plants of Echinocactus

DATE	PLANT NO. 21 (FULL SUNLIGHT)		PLANT NO. 22 (FULL SUNLIGHT)		PLANT NO. 23 (FULL SUNLIGHT)		PLANT NO. 24 (DIFFUSE LIGHT)		PLANT NO. 25 (DIFFUSE LIGHT)		PLANT NO. 26 (DIFFUSE LIGHT)	
	Weight	Per cent of original weight	Weight	Per cent of original weight	Weight	Per cent of original weight	Weight	Per cent of original weight	Weight	Per cent of original weight	Weight	Per cent of original weight
	kg.		*kg.*		*kg.*		*kg.*		*kg.*		*kg.*	
June 24	17.0		14.0		19.2		19.52		26.55		13.00	
July 2	15.7	92.4	13.2	94.3	17.9	93.2	19.24	98.5	26.25	98.9	12.81	98.5
July 9	15.1	88.8	12.6	90.0	17.3	90.1	19.12	97.9	26.02	98.0	12.75	97.7
July 17	14.2	83.5	12.2	87.1	16.5	85.9	19.02	97.4	25.93	97.7	12.68	97.5
Aug. 1	13.3	78.2	11.3	80.7	15.9	82.8	18.82	96.4	25.68	96.7	12.52	96.3
Aug. 22	11.8	69.4	10.1	72.1	14.5	75.5	18.61	95.3	25.39	95.6	12.40	95.4
Sept. 12	11.0	64.7	9.4	67.1	13.6	70.8	18.46	94.6	25.18	94.9	12.25	94.2
Oct. 14	10.1	59.4	9.1	65.0	13.1	68.2	18.24	93.4	24.94	93.9	12.06	92.8
Nov. 2	9.7	57.1	8.8	62.9	12.5	64.6	18.19	93.2	24.90	93.7	12.03	92.5
Nov. 30	9.3	54.7	8.4*	60.0	11.9	61.9	18.10*	92.7	24.79	93.4	11.97	92.1
Feb. 15											11.82*	90.9
Feb. 23	8.3*	48.8										
Mar. 3					10.7**	55.7			24.63*	92.8		

*The plant was then analyzed.

**The plant was then placed in the dark room.

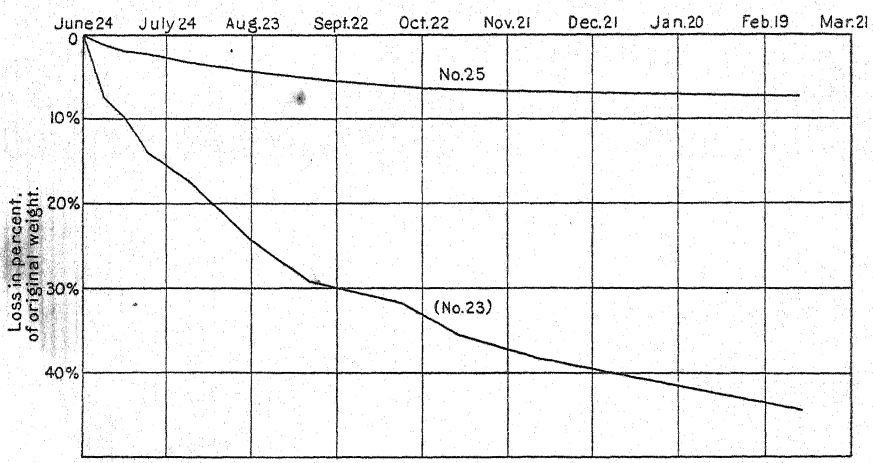

FIG. 3. Tracings illustrating the course of loss in weight of Echinocacti nos. 23 and 25, from June 24, 1914, to March 21, 1915.

The most obvious fact brought out by the data just cited is the relatively great loss of water in the plants exposed in the open, an effect probably due to wind, high temperatures, and other factors tending to increase the evaporative action of the air. The gradual flattening of the curves, indicative of a drop in the rate of water-loss, is also at once appparent. The immediate explanation for this phenomenon must be sought on morphological grounds. The mere increase in concentration of the cell sap would not be sufficient to cause the observed drop in the rate of water loss, although it may be a factor in causing morphological changes. It is a well known fact that within rather wide limits water evaporates from the moist surfaces of colloidal solutions of varying concentration at approximately the same rate as from the surface of distilled water. It is perhaps not out of place to cite an experiment to illustrate this, in which 50 cc. of distilled water and the same amount of a 2 per cent gel of gelatine were used, in similar vessels.

A slightly larger loss from the water surface is noted after eight days at a time when the concentration of the gel has increased more than 20 per cent.

TABLE IV

Evaporation from water surface and from surface of gelatine gel

DATES	TOTAL LOSS FROM GELATINE GEL	TOTAL LOSS FROM PURE WATER
	grams	*grams*
January 8, 2 p.m.	0	0
January 9, 10 a.m.	1.26	1.16
January 13, 2 p.m.	6.92	6.97
January 16, 2 p.m.	11.28	11.44

However, it should be noted that the graph for the rate of loss in weight of the gel is practically a *straight line*, and not a flattening curve, as is the case in the desiccation experiments just recorded. Such results make it apparent that the decreasing rate of loss in weight can not be satisfactorily attributed to increased sap concentration directly, although it is within the range of probability that heightened concentration of the cell-solutions might exert a direct effect upon the membranes, which might, in turn, result in lessened water-loss.

The chemical analyses were confined to those points where changes might be expected as a result of prolonged desiccation and catabolism without repair. As a routine procedure, determinations were made of the dry weight, of the density and total solids of the sap, of the acidity, and of the carbohydrate material available for nutriment. In some cases the protein and fat content and the hydratation or swelling power, were also determined.

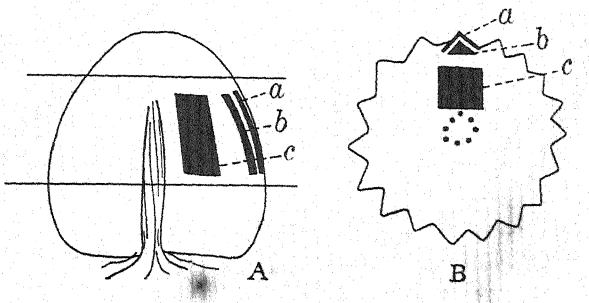

Fig. 4. Diagrams of cross (B) and longitudinal (A) sections of stem of Echinocactus, to indicate parts of the cortex from which samples for chemical and microscopical examination were taken.

In all cases the general plan was followed, of analyzing material from three arbitrarily defined tracts in the plant, which were localized as indicated in figure 4. These regions are designated as *a*, *b*, and *c*, *a* and *b* being in the external cortex so near the general surface that they might be included in the spine-bearing ridges characteristic of this plant, while *c* was taken from a position within a few centimeters of the central cylinder, The last sample (*c*) probably represents nine-tenths of the total mass of the cortex.

The methods employed were all standard ones, easily applied. The dry weight was determined at 100°–105°C, on representative samples of the ground and uniformly mixed material from each of the three tracts. The sap was expressed and strained by means of a fruit press, and its density measured with a Westphal balance. The total solids of the sap were found by evaporation on the water-bath and drying at 100°–105° in the oven. Fehling's solution was used for the sugar determinations. The soluble sugars were estimated after alkalination of the expressed sap and filtration from

precipitated hydrates and carbonates. Reducing sugars were determined directly, by titrating the alkalinized sap directly with Fehling's solution, the copper ferrocyanide test plate method being used for accuracy in the end-point. "Soluble non-reducing sugars" were estimated after one hour's hydrolysis of the alkalinized sap on the water-bath, the material being made 10 per cent acid with HCl. The resulting solution was neutralized, diluted to a standard, and titrated with Fehling's solution, the value for reducing sugars being subtracted from the figure found, to give the correct figure for soluble *non*-reducing sugars. It was noted that the sugar content of the alkalinized and unalkalinized sap after hydrolysis, was the same; that is, that no hydrolyzable carbohydrates were thrown down in the precipitating process.

"Total hydrolyzable carbohydrates" (taken to include all carbohydrates existing in hydrolyzed form) were determined by subjecting 50 g. samples of tissue to four hours hydrolysis with 5 per cent hydrochloric acid, a vigorous ebullition being maintained under the reflux condenser, neutralizing, diluting to 1000 cc., and titrating against Fehling's solution. It is taken for granted that this strength of acid does not appreciably affect cellulose, and that the value obtained roughly measures the carbohydrate which may undergo catabolism in the organism. It was assumed when the experiments were begun that the sugars were chiefly of the hexose type and the analyses described above were carried out to determine reducing sugars, sugars on inversion, and insoluble polysaccharides, it being supposed that the sugars thus determined were dextrose and fructose, sucrose, and starch. However, toward the close of the experiments, analyses made by and at the suggestion of Dr. J. H. Long, who was visiting the Desert Laboratory at the time, brought out the fact that a considerable proportion of the sugars were of the pentose type. Accordingly under "reducing sugars" pentoses as well as dextrose and fructose are probably included, and under "soluble non-reducing sugars" soluble pentosans (as constituents of gums) as well as disaccharides, while the term "total hydrolyzable carbohydrate" probably covers insoluble pentosans and hemicelluloses, as well as starch. While this complicates the chemistry of our problem considerably, the actual interpretation of results from a physiological point of view, is not so much modified as might at first be thought, since we are concerned more with amounts than with kind, bearing in mind, however, the fact that pentoses are typically more stable than hexoses, and perhaps less readily broken down in catabolic processes. Pentoses were estimated in the few cases examined by the Jolles method, by which furfuraldehyde is distilled from the hydrolyzed material and treated with an excess of sodium bisulphite, the amount of excess being determined by titrating back with iodine.[4]

[4] See: Abderhalden, Handbuch der Biochemischen Arbeitsmethoden. 2: 135. 1910.

Acidities are expressed in terms of cubic centimeters of tenth-normal NaOH required to neutralize 1 cc. of the expressed sap (to phenol phthalein). Crude protein was estimated by simple total nitrogen determination, and crude fat by ether extraction. Hydratation, or water-absorption, is expressed in terms of the percentage of the original weight taken up by pieces of tissue of approximately the same weight and surface area, in two hours time in distilled water at equable room temperatures.

Analyses were made of plants of Echinocactus as follows: Nos. 7, 24, 25 and 26, desiccated in diffuse light within the laboratory; nos. 21 and 22, desiccated in full sunlight within the laboratory court; and nos. 34, 35 and 36, normal—i.e., just taken up from their natural habitat. The results of the analyses are summarized in table V. Carbohydrate, protein and fat values are expressed as percentage on the basis of dry weight. The figures given for the sugars are all calculated on the assumption that 10 cc. of Fehling's solution is reduced by 0.05 g. dextrose or pentose. In view of a lack of knowledge, in most cases, of the proportions of pentosan, hemicellulose, and starch separately, corrections for the excluded molecule of water in the dehydrated carbohydrates $(C_6H_{10}O_5)_x$ and $(C_5H_8O_4)_x$ are not made under the heading "Total hydrolyzable carbohydrates;" the figures given in this column represent the total carbohydrate in the hydrated form after hydrolysis, i.e., as $C_6H_{12}O_6$ and $C_5H_{10}O_5$. This fact will be taken into account below, in the consideration of calculations made on the basis of dry weight.

The most interesting feature brought out by table V is the fact that after six years of desiccation, and after a lost of 29.3 per cent of its weight chiefly by water evaporation, Echinocactus no. 7 still averaged about 94 per cent of water, or practically the same as the average of the three normals nos. 35, 36 and 37. The explanation follows easily enough and will be brought out in the consideration of the sugar metabolism immediately following. But at the time of analysis the result was thought startling enough to warrant relocating the basal third of this plant, which had been cut off as usual and discarded, had been lying in the open with the cut surface exposed, for nine days, and had undoubtedly lost a considerable amount of water. A representative sample of this basal portion averaged 91.1 per cent of water. Evidently no serious error had been made in the dry weight determinations, which had been made in duplicate. We were then convinced that changes in the composition of the dry weight, indicative of a loss parallel to the water depletion were to be expected.

The dry weight of samples and the density and total solids of the sap are so closely related with sugar concentrations that they will not be considered separately. It is in the sugars and sugar producing constituents that we find the most marked and most interesting changes, changes which, in turn, explain the variations observed in the dry weight, density and total solids. As might be expected these alterations are at their maximum in the plant starved and desiccated the longest, no. 7.

In no. 7 we find a greatly reduced content of total hydrolyzable carbohydrates, the latter after hydrolysis forming only 11.1 per cent of the dry weigh of the cortex of the plant (sample c). The average content of the normal plants studied, in hydrolyzable carbohydrates, in the same samples (c), which, as has been remarked, represent about nine-tenths of plant, is 26.1 per cent of the dry weight, and one of the plants (no. 35) is probably exceptionally low in this respect.

Something like 15 per cent of the dry weight of the cortex (a little less than 15 per cent as this calculation is in terms of hexoses and pentoses, while a large proportion of the total carbohydrate actually existed as the dehydrated polysaccharides) had disappeared in the course of the long period of low illumination and consequent starvation. In the peripheral tenth of the plant the decrease in sugar content below the normal was not so great. The average content of total carbohydrate in regions *a* and *b* for nos. 34, 35 and 36 was 32.5 per cent of the dry weight, and in the same region in no. 7, 23.3 per cent. Thus, if at the start of its period of starvation, no. 7 had the sugar content in the outer region of a plant with the average sugar concentration found in nos. 34, 35 and 36, it had lost in the course of six years, 9.2 per cent. The average loss for the entire plant, by the same method of calculation, would be about 13 or 14 per cent. Unfortunately we have no way of knowing what the sugar content of no. 7 actually was when it was brought in for experimental purposes. It is to be noted that nos. 34, 35 and 36 were analyzed in early January, when the winter was well under way, while no. 7 came out of the ground on November 7, at the close of the summer—that is, at the close of the period when the conditions for sugar manufacture were at their best, but when disintegration was also very high.

Whatever is true, no. 7 had consumed more than 13 or 14 per cent of its dry weight. It had lost 29.3 per cent of its original weight and still maintained its normal, high proportion of water. A certain proportion of this 29. per cent must have been lost in the combustion of solid material itself, but by far the greater part disappeared in water evaporation. To lose some 29 per cent of its weight by water evaporation and maintain its original proportion of water, it must have lost an equivalent proportion of its dry weight. The loss must have occurred almost entirely in the carbohydrate portion. No loss in inorganic constituents was possible and no loss in protein or lipoidal substances actually took place. The reduction in acidity, to be described later, accounts for a small amount of the loss. The greatly lowered total hydrolyzable carbohydrate content accounts for a very large amount of it, but unless no. 7 was very much richer in this portion of its composition than the normal plants studied in connection with it, not for all. Could other carbohydrates, for example, cellulose and the other substances forming the fibrous system of the plant and ordinarily considered very stable, have taken part? Apparently they did. As will be pointed

out in the discussion of morphological changes, large lacunae were found on section of the plant, scattered through the cortex, where tissue had disappeared completely. A cytolysis involving some of the most stable of the organic substances of the plant's tissues had taken place, leaving the products to follow the fate of the other carbohydrates. It is upon this cytolysis that we must fall back, to explain the balance of dry weight loss which must have paralleled the loss by water evaporation.

So far we have been unable to reproduce this cytolysis experimentally in the laboratory. It is evidently a process requiring a very long time.

As was noted above the drop in total sugar content was not so great in the chlorophyllose tissue as in the deeper parts. No. 7 was not in darkness. A certain amount of photosynthesis was undoubtedly going on enough to furnish the outer parts with something under their normal supply of sugar, but leaving no margin for translocation to the interior.

The drop in the sugar content of no. 7 was very noticeable in the concentration of sugars dissolved in the cell sap. The reducing sugars were very much lower in amount in no. 7 than in the normals, nos. 34, 35 and 36. The soluble non-reducing sugars were also much lower in amount than in nos. 24, 26 and 25, which were "starved" for a much shorter period. This low concentration of soluble sugars, as compared with a normal plant, explains the paradoxical fact that after six years of concentration by desiccation, the sap of no. 7 has actually been lowered in density (see table V), being the lowest recorded in the entire series.

As intermediates between the normals, nos. 34, 35 and 36, on the one hand, and no. 7, on the other, nos. 24, 26 and 25 are not entirely satisfactory. Time had been allowed for a loss in weight not yet amounting to a third of that which had taken place in no. 7, and no appreciable sugar destruction had yet taken place. In fact, the figures would indicate (total hydrolyzable carbohydrate, table V) that a slight concentration was taking place. The change is perhaps not unaccountable on the ground of individual variation.

Comparing nos. 24, 26 and 25 with the normals, nos. 34, 35 and 36, however, we are struck by two other points. The first of these is in the distribution of the reducing sugars. Normally these are found in greatest amount in the outer tissues, where they are being formed, and in progressively decreasing concentration as the center is approached. In nos. 24, 26 and 25 the reverse condition is observed. The concentration is least in the exterior and progressively increases as the center is approached. This is probably to be referred to the slow rate of photosynthesis when the plants were brought indoors. Such an effect would naturally soon be realized if the normal movement of food material toward the interior kept up for any length of time after the rate of food production in the external layers was so greatly lowered. In connection with the change in distribution of reducing sugars,

a drop from the normal in their total amount is also noticed. The second point of departure from the normal observed in nos, 24, 26 and 25 is in the soluble non-reducing sugars. The latter apparently exist in low concentration, or, at times, not at all, in the sap of normal plants. They are present in considerable concentration in the sap of all of the desiccating plants. The fact that they are present in really large amount in the sap of no. 22, a plant desiccated in full sunlight and obviously laying up sugars, and in much less amount in no. 21, a similar specimen, which was rapidly breaking up, however, suggests that they are products of anabolism rather than catabolism.

Nos. 22, 21 and 23 were individuals which were desiccated in full sunlight, that is, under such conditions that they could manufacture sugars. Of these, nos. 22 and 21 were analyzed after periods of desiccation of five and eight months respectively, and no. 23 was put away in a dry dark-room to be studied at a later date for the effect of a lowered rate of water evaporation accompanying a destruction without formation of sugars. The most noticeable features in the analytical results for no. 22, were the high density of the sap and its high content of non-reducing sugars, just touched upon above. The latter, combined with the concentration of dissolved salts occurring, accounts for the former. The laying up of soluble non-reducing sugars was sufficient to cause a marked rise in the proportion of the dry weight present as hydrolyzable carbohydrate. The dry weight had been raised by the desiccation in proportion to the water lost. No. 22 was to all appearances in healthy condition in all respects except water content, and would probably have survived had it been returned to the soil.

No. 21, on the other hand, had possibly suffered to a point where it could not be revived. It was in a state of low turgor, and the tissues of the side most exposed had collapsed. Everything indicates that it was breaking up rapidly. In spite of a water-loss exceeding that of no. 22 by 11.2 per cent, the density of the sap of the cortex of no. 21 was the lower of the two. The total solids of the sap were considerably lower than might have been expected. Determinations of total solids were not made for no. 22, but figures for a comparable specimen are available. Echinocactus no. 10 of a former series (MacDougal [12]) had been similarly exposed, and at the time of analysis had practically the same cortical sap density as no. 22 (1.035), and a total solid content of 7.1 per cent. It seems at first rather surprising that no. 21, which had lost more water than either no. 10 or no. 22 should have a lower sap density and a lower content of dissolved material. The explanation lies in the greatly reduced sugar content of the sap of no. 21. Comparison of the protocols for nos. 22 and 21 shows that both the reducing and soluble non-reducing sugars exist in much lower concentration in the sap of the latter. The total hydrolyzable carbohydrate is also much lower in no. 21. Just why this condition should occur in a plant afforded full opportunities for the

manufacture of sugars, at least as regards illumination, is not easy to say. The chlorophyllose tissue still bore a healthy appearance to the unaided eye. However, the carbohydrate content, so low compared with that of its fellow no. 22, and the higher acidity, would seem to indicate that a rapid combustion was taking place, with nothing like adequate repair. This consideration probably explains the phenomenon noted by MacDougal [12] in a similar case. Echinocactus no. 10 dried out in the open for seven months, losing 33.3 per cent of its weight, and at the time of analysis had a content of dissolved solids of 7.1 per cent. No. 13 of the same series dried out in the open for thirteen months, lost 48.3 per cent of its weight, and at the time of analysis had *a total dissolved solid content of only 3.7 per cent.* Sugar determinations were not made. Had they been made it is likely that a greatly reduced sugar content would have been found in the sap of no. 13. Like no. 21, no. 13 was probably rapidly breaking up. Both plants were, in all likelihood, in advanced stages of starvation.

In connection with carbohydrate metabolism, we should consider some of the intermediate products of the destruction; viz., the organic acids. In the light of recent work, we do not expect to find an increase in acidity necessarily accompanying a concentration of the sap. The effect of light and warmth in destroying the acids accumulating in the sap of succulents from catabolizing sugars, has been measured by Richards,[5] and the course of destruction has been followed in detail by Spoehr.[6] In the course of the experiments recorded here very high acidities were never encountered. The range of acidity of the sap of Echinocacti at the season when nos. 21–26 were taken up from the ground (summer, 1914) was followed carefully,[7] and the average at the time of day when nos. 21–26 were analyzed later (8–10 a.m.) was found to be as follows: *a*, 0.297; *b*, 0.272; *c*, 0.196.

The specimens from the same tracts in Echinocactus no. 24, analyzed in the late fall, are below this average in acidity, and this in spite of the fact that for five months no. 24 had not been getting as much illumination as previous to the commencement of its desiccation—a condition favorable to rise in acidity. However a considerable variation is manifested in normal plants, and it is possible, too, that the explanation lies in the event of a generally lowered metabolism in consequence of the cutting off of the water supply. In nos. 26 and 25, however, which were analyzed some months later, a progressive rise in acidity is noticed. It is altogether probable that this is due to the prolonged low illumination and colder temperatures prevailing. Between September 1 and December 1, temperatures of the air surrounding the plants varied between 95° and 65° F., and between 65° and 40° F. between

[5] Richards, H. M., Respiration and acidity in plants. Carnegie Inst. Wash. Pub., Washington, 1915. (*In press.*)

[6] Spoehr., H. A., Photochemische Vorgänge bei der diurnalen Entsäurung der Succulenten. Biochem. Zeitschr. 57: 94–111. 1913.

[7] Long, E. R., Acid accumulation and destruction in large succulents. Plant World, 1915. (*In press.*)

the latter date and March 1. Combined with the lower temperatures of the latter period was the increased amount of darkness, which in the winter of 1914–15, a very rainy one, was unusually great. Thus, the destructive influences of illumination and warmth being removed to a greater extent than previously, conditions favorable to a rise in acidity are at hand, and we are not surprised to see this rise actually taking place as noted in Echinocacti nos. 26 and 25. (See table V.)

The lowest acidities recorded anywhere in the entire series, were in Echinocactus no. 7, desiccated in diffuse light more than six years. It is not difficult to explain this. As emphasized above, the sugar content of this specimen had reached a very low state, and as acidity under such conditions is apt to be roughly parallel to the total sugar—a fact illustrated in the protocols for nos. 7, 24, 26 and 25—we should expect an accompanying reduction in acidity, provided a sufficient length of time was allowed for the agencies destructive to acidity to bring about a state of equilibrium. No. 7 had probably long since passed through the stage of acidity concentration recorded in nos. 26 and 25, and at the time of analysis was evidently in a final stage of minimum metabolism.

It would be hazardous to attempt to draw conclusions from the acidity values of the other cacti represented in the tables. These were all exposed to full sunlight and were undoubtedly undergoing great diurnal variations in acidity. In the routine procedure of analysis the acidity of these plants was determined about 11 a.m., after some hours of sunlight, and accordingly comparisons cannot fairly be made with the specimens kept indoors under more equable conditions of light and temperature.

It would not be unnatural to suppose that the tissues of a plant which had steadily dried out for more than six years, would have an abnormally high power of absorbing water. Certainly the osmotic pressure of the cells would be raised by the concentration of dissolved salts. However there is another factor to be taken into account, which has been receiving a steadily increasing prominence in the literature of recent years. This is the feature of colloid hydratation.[8]

It has been found that the tissues of platyopuntias take up water readily from neutral and alkaline media, and somewhat less readily from an acid surrounding. Echinocactus has not been investigated, but it is likely that conditions are similar. In Echinocactus no. 7 we have a balanced state of affairs, an increased power of absorption, due to a higher osmotic pressure resulting from salt concentration, and a greatly lowered carbohydrate content (see table V) making for a weaker absorbing power, together with a low acidity, which may or may not be favorable to hydratation. Echinocactus no. 25, which had dried out relatively little (7.2 per cent loss in weight as

[8] Long. E. R., Growth and colloidal hydratation in cacti. Bot. Gaz. 59: 491–497. 1915.

TABLE V

Results of analyses of tissues of Echinocactus

PLANT NO. AND TREATMENT	TOTAL LOSS, PER CENT OF ORIGINAL WEIGHT	TISSUE SAMPLE	DRY WEIGHT, PER CENT OF TOTAL WEIGHT	SAP DENSITY (WATER = 1.00)	TOTAL SOLIDS IN SAP, PER CENT OF TOTAL SAP WEIGHT	SAP ACIDITY, $\frac{N}{10}$	TOTAL HYDROLYZABLE CARBOHYDRATE, PER CENT OF TOTAL SOLIDS	TOTAL REDUCING SUGARS IN SAP, PER CENT OF TOTAL SAP WEIGHT	TOTAL NON-REDUCING SUGARS IN SAP, PER CENT OF TOTAL SAP WEIGHT	HYDRATATION (WATER ABSORBED), PER CENT OF ORIGINAL WEIGHT OF SAMPLE
No. 7 Desiccated in diffuse light 6 yrs. 1 mo. Analysis begun Dec. 8, 1914.	29.3	*a*	9.50	1.010	.....	0.144	22.3	0.09	0.11	9.4
		b	7.96	1.008	.....	0.104	24.2	0.06	0.10	11.6
		c	5.75	1.013	.....	0.148	11.1	0.04	0.06	11.2
No. 24 Desiccated in diffuse light 5 mo. 9 da. Analysis begun Dec. 3, 1914.	7.3	*a*	10.9	1.018	.....	0.212	31.1	0.16	0.30	.....
		b	9.3	1.018	.....	0.192	28.6	0.21	0.25	.....
		c	8.4	1.020	.....	0.156	23.8	0.31	0.46	.....
No. 26 Desiccated in diffuse light 7 mo. 23 da. Analysis begun Feb. 15, 1915.	9.1	*a*	9.6	1.020	4.05	0.220	32.9	0.10	0.80	.....
		b	7.7	1.020	3.84	0.212	27.6	0.18	0.65	.....
		c	7.5	1.020	3.87	0.204	25.6	0.50	0.50	.....
No. 25 Desiccated in diffuse light 8 mo. 6 da. Analysis begun Mar. 2, 1915.	7.2	*a*	10.2	1.019	3.51	0.588	33.8	0.05	0.62	9.3
		b	7.5	1.017	3.12	0.460	33.3	0.14	0.46	13.2
		c	6.0	1.016	3.04	0.212	28.8	0.33	0.31	10.9
No. 22 Desiccated in full sunlight 5 mo. 6 da. Analysis begun Dec. 1, 1914.	40.0	*a*	14.3	1.016	.....	0.244	44.3	0.15	1.28	.....
		b	13.3	1.027	.....	0.208	44.2	0.13	1.48	.....
		c	11.3	1.034	.....	0.156	43.4	0.10	2.67	.....
No. 34 Not desiccated. Removed from ground 2 da. before analysis, which was begun Jan. 11, 1915.	.....	*a*	5.84	1.013	.....	0.172	32.3	0.53	0.14	.....
		b	4.20	1.011	.....	0.156	35.7	0.42	0.03	.....
		c	3.55	1.011	.....	0.128	29.6	0.10	0.05	.....

TABLE V—Continued

PLANT NO. AND TREATMENT	TOTAL LOSS, PER CENT OF ORIGINAL WEIGHT	TISSUE SAMPLE	DRY WEIGHT, PER CENT OF TOTAL WEIGHT	SAP DENSITY (WATER=1.00)	TOTAL SOLIDS IN SAP, PER CENT OF TOTAL SAP WEIGHT	SAP ACIDITY, $\frac{N}{10}$	TOTAL HYDROLYZABLE CARBOHYDRATE, PER CENT OF TOTAL SOLIDS	TOTAL REDUCING SUGARS IN SAP, PER CENT OF TOTAL SAP WEIGHT	TOTAL NON-REDUCING SUGARS IN SAP, PER CENT OF TOTAL SAP WEIGHT	HYDRATATION (WATER ABSORBED), PER CENT OF ORIGINAL WEIGHT OF SAMPLE
No. 35										
Not desiccated, removed from ground 9 da. before analysis which was begun Jan. 18, 1915.	.....	*a*	8.12	1.018	.....	0.204	28.6	1.11	0.08	.....
		b	6.23	1.018	.....	0.204	29.1	0.91	0.05	.....
		c	5.42	1.018	.....	0.220	19.7	0.39	trace	.....
No. 36										
Not desiccated, removed from ground 12 da. before analysis, which was begun Jan. 21, 1915.	.....	*a*	10.18	1.018	.....	0.172	35.0	1.13	0.03	.....
		b	8.77	1.017	.....	0.140	34.5	1.28	0.0	.....
		c	8.66	1.016	.....	0.136	29.1	1.02	0.0	.....
No. 21										
Desiccated in full sunlight 8 mo. Analysis begun Feb. 23, 1915.	51.2	*a*	17.8	1.028	5.48	0.400	25.3	0.09	0.58	.....
		b	15.7	1.028	5.40	0.260	30.1	0.03	0.56	.....
		c	12.9	1.028	5.49	0.192	25.8	trace	0.37	19.3
No. 23										
Desiccated in full sunlight 8 mo. 10 da. placed in dark room Mar. 6, 1915.	44.3									

against 29.3 per cent in no. 7) and had a high content of hydrolyzable carbohydrates—for the most part probably substances of colloidal nature—was examined as to its swelling power for comparison. Not a great deal of difference was found (see table V). The swelling of no. 7 is slightly greater in the case of *a* and *c* (fig. 4), and appreciably less in the case of *b* (fig. 4). It seems likely that, as compared with no. 25, the tendency to increased absorption in no. 7, due to a higher osmotic pressure, was practically counterbalanced by the much lower colloidal content and accordingly sections from the two plants swelled to about the same degree. In Echinocactus 21, which had dried out in the sunlight to such an extent that it had lost half its water,

and presumably had a high absorbing power on both increased osmotic pressure and high colloid content, the imbibition of water by the deep cortex (*c*) under similar conditions of time and temperature, was 19.3 per cent or nearly twice that of nos. 7 and 25.

Nitrogenous and lipoidal constituents form so small a proportion of the tissue of the Echinocactus that changes in their amount are of relatively little importance in the gross effects of catabolism, and for this reason they were not taken into account extensively in our conduction of the problem, which was concerned with the greater, more readily visible changes. That alterations, however, in their amount or character, as a result of the rigorous conditions to which the plants may be exposed, would have great effect upon the course of the rest of the metabolism, is altogether likely, for in all probability the substances concerned are essential to the life of the plant in nuclear metabolism and may play a part in the permeability of the membranes. On the other hand, these substances would be the last constituents

TABLE VI

Contents of crude protein and of crude fat in tissues of desiccated and normal Echinocactus

ECHINOCACTUS	TISSUE SAMPLE	CRUDE PROTEIN	CRUDE FAT
		per cent of total dry weight	*per cent* of total dry weight
No. 7, desiccated 6 yrs. 1 mo.....	*a*	2.50	0.75
	b	2.63	0.80
	c	0.81	0.97
No. 35, Normal.....	*a*	2.31	1.11
	b	2.31	0.93
	c	0.50*	1.01

*Approximate.

to break down and disappear under conditions of starvation without adequate repair. Therefore it is not surprising that no distinct changes were noted on subjecting a desiccated and a normal specimen to protein and lipoid analysis. The slight differences found may be assumed to lie within the ordinary limits of variation existing among normal plants. The data of protein and fat content, as obtained from these analyses, are presented in table VI.

It should be noted that the value of the protein determinations here given must be discounted somewhat by the fact that a certain amount of nitrate (which would be the end product of protein catabolism) would be included in the "Total N" determination. The greater part would probably go off as nitric acid in the course of a Kjeldahl analysis.

The protein of *c*, Echinocactus no. 35, was not determined with absolute accuracy because of an accident in the titration.

From the chemical data recorded above we can reconstruct with some accuracy of detail the story of starvation in a desiccating succulent. When

one of these massive plants is deprived of its water supply and brought into diffuse light where its photosynthetic activity is greatly lowered, the first effect is a simple loss of water, the rate of which, however, gradually falls. The first change noticeable in the food material is in the concentration and distribution of the soluble sugars. The rate of production of reducing sugars has notably decreased, and those present in the sap at the initiation of the starving period are removed by translocation and catabolism. A distribution of these sugars which is the reverse of the normal occurs; the concentration becomes greatest in the interior and progressively less as the surface is approached. At the same time soluble non-reducing sugars, ordinarily present to slight extent only in the sap of normal plants, appear in appreciable concentration, in a manner not easily explainable. As the period of low illumination is continued, the acidity begins to rise.

Sometime after the first year the content of carbohydrate available for nutriment begins to drop. The concentration of dissolved sugars is probably the first to be lowered, and is followed by a breaking up of the insoluble polysaccharides at a rate exceeding their restoration. A resultant fall in the dry weight roughly parallel to the water loss is thus established, of such effect that the plant may maintain its normal high proportion of water or even exceed it. Photosynthesis continues to a certain extent in the outer cortex, in which the sugar content does not vary greatly from the normal. But the drain is felt in full force in the interior in the great body of the plant. The original distribution of reducing sugars, in which the latter steadily decrease in amount as the center is approached, is restored. Only their concentration is greatly reduced. At the same time the amount of soluble non-reducing sugars is lowered. Coincident with this lowering of sugar concentration, in spite of continued water evaporation, the sap density drops to a point even below that of a normal plant.

As the amount of carbohydrate capable of undergoing catabolism decreases, the acidity, after its preliminary rise in response to the removal of light, begins to fall, not because its rate of destruction is increased, but because its production through the catabolism of sugar is so greatly lowered. A point is reached where even in diffuse light, acid destruction proceeds more rapidly than acid formation. Eventually cytolysis begins. During all this time the protein and fat content remains normal.

After six years of desiccation and a loss of a quarter of its weight, we may have a plant with a proportionate content of water the same as that of a normal plant, in the neighborhood of 94 per cent, and with a sap density not differing greatly from the normal. But a chemical analysis shows that the water ratio has been maintained only because the carbohydrate food supply has burnt at a rate parallel to the water-loss, and has become greatly lessened.

Consequently the tissues have no increased power of hydratation, for the

colloid substances active in the absorption of water by normal tissues, have been to a large extent removed.

Desiccation of Platyopuntias

The principal features of desiccation of the flattened joints of the opuntias of this region being known to be something different from those presented by the globose Echinocacti, it was deemed essential to follow a number of these plants through the earlier stages of depleted water-balance in order to compare the variations in weight and water-content with those discussed in the previous section. The material consisted of twenty-four turgid joints of *Opuntia discata* taken from two plants growing near the chemical building of the Desert Laboratory on September 28, 1914. The separate joints were

TABLE VII

Water loss of desiccating opuntias

DATE	6 PLANTS OUTDOORS, UPRIGHT	6 PLANTS OUTDOORS, FLAT	6 PLANTS INDOORS, UPRIGHT	6 PLANTS INDOORS, HORIZONTAL
	per cent of original weight	*per cent* of original weight	*per cent* of original weight	*per cent* of original weight
September 30	100.0	100.0	100.0	100.0
October 2	97.1	97.7	98.2	97.
October 5	96.1	96.7	97.1	96.7
October 9	93.4	95.1	95.9	95.6
October 14	91.1	93.2	94.7	94.0
October 19	88.6	91.9	93.3	92.8
October 26	86.2	90.2	92.2	91.7
November 2	85.0	89.2	91.3	90.7
November 16	83.0	87.7	89.8	89.6
November 30	82.0	86.8	88.7	88.5
December 30	80.7	85.7	87.5	87.5

taken from terminal portions of the plant and were cut cleanly at the base in such manner as to be as nearly equivalent as possible in evaporative capacity. Six were arranged on a wooden support in an upright position in the open, exposed to the full illumination, the planes of the joints being north and south; six were placed near these but in a horizontal position, being turned once every week so that the two sides were alternately uppermost; six were similarly placed in an upright position in the diffuse light in the middle of the largest room in the main building of the Desert Laboratory, and six in a horizontal position in the same place. Weights were taken as given in table VII, and as graphically illustrated in figure 5.

The most noticeable feature brought out by comparison with the data obtained from the Echinocacti is that the difference in the rate of decrease in weight between plants in the diffuse light and those in the open at much

higher temperatures and exposed to full sunlight is comparatively small at all times, being practically identical after the first thirty-five days of exposure. At this time Echinocacti were losing weight at a rate four times as great in the open as in the shaded room.

It is to be noted that platyopuntias drying out indoors lose water more rapidly than Echinocacti under the same conditions, while platypuntias desiccating in the open in full sunlight lose water considerably less rapidly than Echinocacti drying in the open. The latter fact may be of some ecological significance in explaining the vastly wider distribution of the platyopuntias.

The effect of *position* upon water loss, is well shown in table VII. Platyopuntias desiccating in the upright position, exposed in the open, lose water more rapidly than similar joints lying horizontal. This is probably an effect of the different amount of illumination operating in each case. The plants set up-

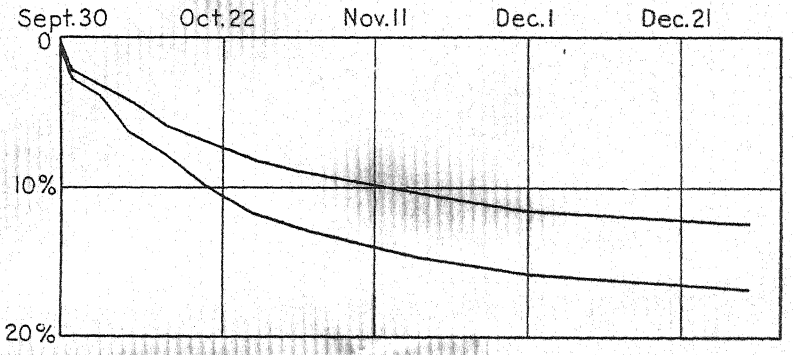

FIG. 5. Tracings illustrating course of loss in weight of a platyopuntia, in terms of original weight. The upper line expresss average of 12 joints desiccating in diffuse light of shaded room, the lower line expresses the average of 12 joints desiccating in the open near the Desert Laboratory.

right were exposed to sunlight and sky radiation on both sides throughout the day, while in the plants lying flat one side was naturally shaded. It should be noted that the frames were so constructed that the joints were as much exposed to the air on one side as upon the other. As might be expected this effect of position does not appear in the results for the plants desiccating indoors, where the factor of illumination is so greatly modified.

Chemical analyses undertaken after three months of desiccation were confined to acidity and total hydrolyzable carbohydrate determinations. The latter were made in the same manner as for the Echinocacti.

The presence of slimes renders the expressing of the sap for determination of acidity very difficult, and a method of alcoholic extraction was used, a weighed amount of the finely ground material being suspended in a Soxhlet extraction shell in a wide-mouth flask attached to a reflex condenser, in

such a manner that the condensing alcohol from 50 cc. of boiling alcohol in the bottom of the flask drips through the material to be extracted. The alcoholic extract is titrated. The acids in question are all alcohol soluble. The possibility of error in this method was found to be so great as to render its general use inadvisable. The differences noted in this work however are greater than the possibility of error and are in accord with the expectancies as established by the result of Richards and Spoehr. The results of these acid determinations and those of parallel determinations of total hydrolyzable carbohydrates, are given in table VIII.

A rise in the dry weight in the desiccated joints was noted, which was greatest in those which had been exposed in the open, a rise in acidity in joints desiccating in diffuse light, a drop in acidity in joints desiccating in the open, and a small fall in the hydrolyzable carbohydrate value in desiccating plants, the same for the two conditions of desiccation. The dropping of the carbohydrate figure in the joints desiccating in the open is perhaps to

TABLE VIII

Analyses of desiccating platyopuntias

DESCRIPTION OF MATERIAL	DRY WEIGHT	ACIDITY, cc. $\frac{N}{10}$ NaOH PER G. OF TISSUE	TOTAL HYDROLYZABLE CARBOHYDRATES
	per cent of fresh material		*per cent* of dry wt.
Average of 2 normal joints. Analyzed Jan. 6	12.1	0.83	37.9
Average of 3 joints desiccated indoors 3 mos. Analysed Dec. 31	21.7	1.30	33.1
Average of 3 joints desiccated outdoors 3 mos. Analyzed Dec. 31	25.3	0.52	33.1

be referred to the fact that the chlorophyllose structure was being slowly affected, for the joints were becoming yellowish and photosynthetic power was correspondingly lowered. It is rather surprising that the joints which had been resting in weak light for three months still showed at the end of that time so high a content of hydrolyzable carbohydrates. A slow rate of catabolism is apparently indicated.

The rise in acidity in the plants which had desiccated indoors is probably a result of the long period of low illumination. The drop in acidity in the joints exposed in the open is perhaps a sequence of the falling carbohydrate value. The figures for the two normal joints upon which these assumptions of fall and rise in acidity are made, is fairly typical for plants at the time of day at which the analyses were made, as it is in close agreement with a large number of other observations made in this laboratory.

The hydratation or swelling power of two of the joints was determined at the beginning and at the close of the experiments. Joints no. 7 and no. 19,

desiccated outdoors and indoors, respectively, were chosen for this purpose. On September 30 plugs 2 cm. in diameter were cut from each with a cork borer, placed in distilled water in the laboratory equable temperature dark room, and the swelling after twenty-seven hours noted. The wounds healed, and the joints dried out subsequently similarly to the others. On January 7 the experiment was repeated on the same joints. Table IX summarizes the results, imbibed water being measured in per cent of the weight of the imbibing tissue.

Swelling values, not greatly different at the start, diverge widely at the close of the experiment, under the two sets of conditions. Several factors contribute to the change. Chief of these are, osmotic pressure, the amount of dry material involved, and the acidity of the plant sap. From the results of table VIII we may assume that the acidity of no. 7 was higher than

TABLE IX

Hydratation of tissue from desiccating platyopuntias

DESCRIPTION OF MATERIAL	SWELLING, PER CENT OF ORIGINAL WEIGHT	
	Sept. 30	Jan. 7
No. 7, desiccated 3 mos. outdoors. Had lost 24.7 per cent of its original wt.....	55.4	84.6
No. 19, desiccated 3 mos. indoors. Had lost 16.8 per cent of its original wt.....	51.1	45.6

that of no. 19, and that the acidity of the latter (desiccated three months in diffuse light) was considerably higher than normal. We know that acidity is an inhibiting factor in water imbibition by the tissues of platyopuntias.

Accordingly, we are justified in summarizing as follows: nos. 7 and 19 swelled to approximately the same degree on September 30 because they were similar, normal joints. On January 7, no. 7 took up water in vastly greater proportions because it had dried out to a greater extent than no. 19, with the result that the osmotic pressure of its cells was raised, and the relative amount of colloidal material active in hydratation was increased, and also because the acidity of its sap was lower than that of no. 19. It is probably the latter feature, the high acidity, which is responsible for the decrease in swelling power below its normal, observed in no. 19, in spite of three months of desiccation. A slightly lowered sugar content is perhaps an additional factor in this decrease. (Long [15].)

THE EFFECT OF DESICCATION ON THE STRUCTURE OF ECHINOCACTUS WISLIZENI

J. G. BROWN

The effect of desiccation on the structure of living tissues has been studied chiefly by zoologists who were interested in the ability of the rotifers and other invertebrates to survive extended periods of drouth. The results of these investigations, especially those on the cytological changes induced by desiccation in the various organs of rotifers, have been briefly reviewed by Hickernell in a recent paper[9] in which the author also presents the results of his own investigations. Hickernell's paper is especially interesting to botanists for he compares the changes found in tissues of the rotifer with those occurring in plant cells enduring similar dry conditions. He finds that the changes in cell organization in the various tissues of the rotifer are nearly uniform. The cytoplasm shrinks, ceases to exhibit a normal arrangement of its particles, and loses its ability to hold stains. The nucleus is even more sensitive to water starvation. Granular material evidently derived from the large karyosome of the normal nucleus, migrates to the peripheral region of the nucleoplast where it forms a thick ring, so that the relative positions of granular element and ground substance are reversed. No decrease in the size of the nucleus is observable. In the cells recovering from desiccation, nuclei show a reformation of the karyosome and consequent reduction of the granular ring. These changes in the rotifer are compared with those found in cells of desiccated maize embryos and they are found to be analagous. Recovery of desiccated cells in the rotifer is marked by changes almost the reverse of those following drying, according to Hickernell.

Although the periodic drying of plants, such as certain algae, pteridophytes and liverworts, and their subsequent recovery is even more remarkable than the ability of the rotifer to resist desiccation, there is a paucity of literature on the cytological changes in the cells of these plants resulting from conditions of drouth. Pfeffer[10] comments on physiological phenomena attending desiccation, but mentions no changes in cell structure. In this part of the present paper an attempt is made to present a clear picture of the cellular changes in plant tissues, at least so far as the tissues of a succulent are concerned, that result from water starvation.

The chief object of the investigation was to determine changes in structure brought about by continued deprivation of water, and to discover any evidence of recovery on the part of the plant when, after a period of desiccation it was again subjected to normal out-of-door conditions. Preliminary to the study of the desiccated material a careful examination of the structure of the normal plant was made.

[9] Hickernell, Louis M., A preliminary account of some cytological changes accompanying desiccation. Biol. Bull. 27: 333-342. 1914.

[10] Pfeffer, W., Physiology of plants, translated by A. J. Ewart. 3: 249-256. Oxford, 1903.

MATERIAL STUDIED AND ITS PREPARATION

The material used in this study consisted of (1) normal tissue, (2) tissue from the stem of a cactus which had been deprived of water supply for six years, (3) tissue from a plant deprived of water for ten months, and (4) tissue from a plant which had been deprived of water for forty-two months and then transplanted. All of the material with the exception of the normal tissue, was furnished by Dr. D. T. MacDougal of the Desert Laboratory who has given a detailed description of the conditions surrounding the plants from which it was obtained in another section of this publication. This material had been killed and run through the alcohols up to 80 per cent. The normal material was collected by the writer in the neighborhood of the Desert Laboratory, killed in chrom-acetic mixture, and run through the alcohols in the same manner in which the desiccated material had been treated. The usual difficulty in sectioning cactus stems because of the presence of numerous crystals of calcium oxalate was experienced, but sectioning razors were kept sharp, and a fairly good series of slices of each plant was finally obtained. Some of the sections were stained in safranin-orange G, some in safranin-Delafield's haematoxylin, some in magdala red; some were treated which chloriodide of zinc and examined while still fresh, and others were treated with iodine. Pieces of the stems studied were taken from apical, middle and basal regions, and comparison was made in each case with tissues from approximately identical locations.

STRUCTURE OF THE NORMAL ECHINOCACTUS

Although the structure of Echinocactus has been described by Schleiden,[11] Preston,[12] and others, it seems advisable to give here a short description of the structure of the normal tissues most directly concerned, in order to facilitate comparison.

The integument consists of a more or less thickened cuticle, an epidermis, with thick external wall and thin vertical and inner tangential walls, and a hypoderm of four or five layers of stone cells with deep pits. The entire integument was found to average 200 micra in thickness in pieces taken 30 cm. below the apex of the stem from parts of ridges half way between the areoles. Of this thickness the cuticle made up 9 micra and the epidermis from 35 to 43 micra. The outer walls of the epidermal cells measured 11 micra in average thickness. The epidermal cells were well supplied with protoplasm and their nuclei measured 6.5 micra in diameter. Stomata had guard cells with anterior walls that averaged 2.0 micra in their thinnest portions and gradually thickened to 3 or 4 micra at their borders near the outer

[11] Schleiden, M. J., Beiträge zur Anatomie der Cacteen. Mem. Acad. Sci. St. Petersburg. IV, 4: 335-380. 1841.

[12] Preston, Carleton E., Southwestern Cactaceae. Bot. Gaz. 30: 348. 1900. Also 32: 39. 1901.

guard cell ridges. Posterior walls of guard cells were 1.2 micra thick. Division of the epidermal cells in a tangential direction results in the formation of new epiderm and hypoderm as described for *Echinocactus eyriesii* by Schleiden. Of the two layers of cells thus formed the lower becomes the phellogen.

Next underneath the hypoderm lies the extensive palisade region, which reaches inward for a distance of 2.5 mm. The cells of the palisade are either elongated cylinders or are barrel-shaped, and are connected at the ends to form long columns. The arrangement is such that the intercellular space is great in extent and the tissue extremely loose, for the cells of each column are in contact laterally with cells of adjacent columns throughout a very small part of their surface area. The shortest cells of the palisade region measured 57 micra in length, the longest ones 275 micra. Single walls were 1 to 2 micra in thickness. All cells were fairly rich in protoplasm considering their size. Chloroplasts were numerous and arranged along the sides of the cells (plate I, fig. 3). Nuclei were lenticular and 32 to 35 micra in length (plate II, fig. 1). The nuclear membrane was thin. In addition to the one nucleolus, numerous granules uniform in size and about one-tenth as large were present. The nucleus was usually located at one side of the cell.

The palisade region of the cortex changes rather abruptl yinward into a tissue composed of rounded cells (plate II, fig, 3), which in the ridges of the stem extends entirely across from one palisade region to another. The cell wall surfaces in contact were here greatly reduced in area, a portion of the cell often extending outward in the form of a short tube to meet a similar process from an adjoining cell. Single walls were from 1 micron or less to about 2 micra in thickness. Plastids appeared occasionally in the cytoplasm. Nuclei were round or oval in form and were 6 to 12 micra in greatest diameter. The cells varied in amount of protoplasmic content, some having only a very delicate network of strands in addition to the layer of cytoplasm around the wall, and others having a much coarser reticulum from wall to wall; none was found in which the protoplasm next to the wall could not be easily recognized with the high power dry lens of the microscope. The location of these cells of the outer cortex and also that of the other regions previously described, is shown in plate I, figure 1.

Deeper cortex consisted of cells mostly rectangular in cross-sections of the stem. These cells varied as much in size as the cells of the outer cortical tissue, but were more compact. The walls were slightly thicker than those of the cortical cells lying near the palisade region, but in amount of protoplasm and in form, size, and structure of the nuclei, the cells of the two regions of the cortex were alike.

In the medulla the cells were mostly irregularly six-sided or rectangular in cross-sections of the stem. They varied in size like the cells of the deep

cortex, and had walls 3 to 9.5 micra thick. The cytoplasm varied as in the cortical cells, from a thin sheet closely attached to the walls to a reticulum of loose strands reaching from wall to wall. The nuclei were round or oval in form and about 8 micra in greatest diameter. Their structure was similar to that of the cortical nuclei.

STRUCTURE OF ECHINOCACTUS NO. 7

This plant as previously stated, was without water supply for six years. The integument was about normal in thickness, but the cuticle was thicker than normal, averaging 12 micra. Epidermal cells had external walls 6.4 micra thick, as compared with 11 micra in the normal plant. The nuclei in the epidermal cells were half the size of those in the normal epidermis, averaging only 3 micra in greatest diameter. The cytoplasm formed a thin layer adhering to the external tangential and vertical walls, and a thick layer containing the nucleus on the internal tangential wall. Most of the stomata were partly open; a few had a collapsed appearance. In the latter the cytoplasm of the guard cells was condensed into a small body at the lower side of the cell. Anterior walls of the guard cells averaged 1.3 micra thick in the thinnest portion, and in many cases did not increase much in thickness toward the tangential walls. Posterior walls were 1.6 micra in thickness. Desiccation did not prevent the epidermis from acting in a normal way in the formation of new cork layers (plate I, fig. 2). Epidermal cells observed in the process of division were located in the grooves of the stem. Nuclei in the cells about to divide showed an increase in size as in normal tissue, and the cytoplasm was greater in amount than in the inactive cells of the ridges of the stem.

The palisade region was about 2 mm. thick, 500 micra less than in the normal plant. Many of the cells nearest to the hypoderm, especially those most unsupported by lateral contact with other cells exhibited concave side walls (plate I, fig. 1). Most striking however, was the condition of the protoplasmic contents. In no cell could a layer of cytoplasm be made out entirely around the inside of the cell wall. Every cell examined showed a very small amount of cytoplasm usually restricted to one corner of the cell, in which the nucleus was embedded (plate III, fig. 1). Single cell walls varied from 1.5 to 3.5 micra in thickness. In a few cells the shrunken remains of one or two plastids were visible

The nucleus itself, in the palisade region, was greatly modified in both form and structure. Instead of having the lenticular form found in normal palisade cells, the nucleus was here circular, kidney-shaped, or loaf-shaped. Vacuoles had disappeared from the nucleoplasm and a thickened granular layer had formed in the peripheral portion (plate II, fig. 2). These changes agree with those described by Hickernell for nuclei of dried maize embryos

and rotifers. The greatest reduction in size of nuclei was also noticed in the cells of this tract, some nuclei measuring only 4 micra in greatest diameter and the largest 6 to 8 micra, whereas the normal nuclei of the same region measured 32 to 35 micra.

The most conspicuous effect of desiccation on the structure of Echinocactus was the appearance of lacunae in the cortex. When no. 7 was dissected, irregular spaces from a centimeter or two in length to a size just visible to the unaided eye were found by Dr. MacDougal and Mr. Long (plate I, fig. 4). The lacunae appeared in the greater part of the cortex from the outer median region to the vicinity of the stele. Sections supported the suggestion that the spaces were formed by the hydrolysis of cell walls. The earliest stages in the development of lacunae were best shown in sections made from the tissue of Echinocactus no. 25, the specimen desiccated from June to March. In the inner cortex, cell walls were very irregular in thickness and in places presented a swollen appearance (plate III, fig. 2). Application of chloroiodide of zinc gave the characteristic violet cellulose reaction in the denser portions of the walls, but the color gradually faded out in the more gelatinous material of the wall margins. In later stages the walls presented no structural outline, but appeared to merge into the cytoplasm of the cell when the latter was present. Some walls had entirely disappeared (plate III, fig. 4), and others were almost entirely broken down (plate III, fig. 3, *x*). A few cells bordering the lacunae which had not yet grown large, contained cytoplasm. In these cells the nuclei were much shrunken and evidently about to disorganize (plate III, fig. 4 *a*). Cells bordering the larger microscopic lacunae had collapsed so that often four or more walls appeared in sections to form a ropy mass surrounding the lacunar spaces (plate III, fig. 4, *b*).

Vascular bundles of the stele appeared to be unaffected by desiccation. In the medulla, however, the protoplasmic content of the cells was much reduced in amount and the nuclei were smaller than those of the medulla of the normal plant. Cell walls were irregular in thickness and were disintegrating in spots. Several young lacunae were present in most of the sections examined. The effect of the drouth was less marked in the medulla than in the cortex.

STRUCTURE OF ECHINOCACTUS NO. 25

This specimen had been deprived of water for a period of ten months under conditions similar to those of no. 7. The integument showed a normal thickness of about 200 micra. Its cuticular layer averaged 12 micra in thickness. Epidermal cells were mostly normal in appearance, but a few had a reduced amount of cytoplasm, and in the latter the nuclei exhibited the narrow region of thickening in the peripheral portion of the nucleoplasm. Vacu-

oles had disappeared also and all nuclei of the epiderm were reduced in size, averaging from 3 to 5 micra. Outer epidermal walls in this plant were 12 micra thick. Guard cells were mostly normal, but some had posterior walls thicker than anterior walls.

Palisade tissue was from 1.2 mm. to 2 mm. in thickness. None of the cells presented the shrunken appearance of those in the outer region of the palisade of no. 7. Walls were 1.7 micra thick and were regular in outline. Protoplasm was less abundant than in the normal palisade cells, and chloroplasts only half as numerous. The nuclei were like those of the palisade cells in no. 7, in shape, and were 5 to 7 micra in diameter. Some were non-vacuolated with a narrow, thickened peripheral ring, others were vacuolated.

The outer cortex had the appearance of the corresponding tissue in no. 7, cells being less rounded than normal. Single cell walls were somewhat irregular in thickness, ranging from 1.5 micra to 7 micra. The protoplasm was usually restricted to a layer adhering to the cell wall. Nuclei were 6 to 7 micra in diameter, as compared with 6 to 12 micra in the normal plant.

Median and deep cortex, as previously stated, showed great variation in cell wall thickness, due to swollen parts of walls. The thinnest measured less than 3 micra and the thickest over 20 micra. No lacunae large enough to be seen with the hand lens were found, but a few consisting of the cavities of two or three cells with adjacent intercellular spaces, were observed. Protoplasmic contents of the cells varied in amount, some containing more than the cell shown in plate III, figure 2, others containing as little as the cortical cells of no. 7. A few cells lying close to the phloem of the stele and within 200 micra of cells with swollen walls, contained a varying number of starch grains (plate II, fig. 5). The grains were undergoing digestion, for some were much corroded and others were already broken into small particles.

Medullary cells were similar to the cells of the deep cortex in irregular thickness of walls. No lacunae were present. Many nuclei exhibited the outer granular ring, and vacuoles had disappeared from the nucleoplasm.

STRUCTURE OF ECHINOCACTUS NO. 6

This plant was allowed to desiccate for forty-two months and was then set in the soil in the open. It was examined twenty-two months later. The integument averaged 240 micra in thickness, the increase over the other specimens being partly due to thicker hypoderm. The cuticle was 12 to 14 micra thick and the outer epidermal wall 10 micra in thickness. Epidermal cells were normal in size and amount of protoplasmic contents, and their nuclei had also become normal in appearance. Some of the stomata had guard cells with posterior walls twice the thickness of the anterior walls, and a few guard cells had anterior and posterior walls of the same thickness.

Palisade cells were normal in shape and in amount of protoplasm. Those near the epidermis exhibited about half as many chloroplasts as were present in the normal cells of the same region, but in no. 6 the chloroplasts were congregated around the nucleus instead of being arranged around the entire side wall. The chloroplasts were also smaller than normal. Nuclei were intermediate in size between those of normal palisade cells and those found in the palisade cells of no. 7. They measured 8 to 12 micra in diameter as compared with 32 to 35 micra in the normal Echinocactus and 4 to 8 micra in plant no. 7. Only one nucleus was observed that had taken on the normal lenticular shape, most of them being round or loaf-shaped like the palisade nuclei of no. 7. The outer thickened ring was reduced in size but still present in a few nuclei, in others it had entirely disappeared. Vacuoles were present in many of the nuclei.

The outer cortex, under low power of the microscope, appeared to be like that of the normal plant, but closer inspection showed that walls were irregular in thickness. The variation in the thickness of the walls ranged from less than 2 micra to over 10 micra. Cell contents also varied from a normal amount to that of desiccated tissue. Nuclei varied from 8 to 13 micra in diameter. Some yet possessed the outer granular ring characteristic of the nuclei of no. 7 and exhibited small vacuoles or none at all; others were normal in structure.

The deeper cortex showed very little evidence of recovery from the effects of desiccation. A few nuclei were larger than normal, measuring from 16 to about 23 micra in diameter, and the cells containing such nuclei had an increased amount of cytoplasm over that of neighboring cells with smaller nuclei. Some of the cells contained no protoplasmic contents that could be discerned with the oil immersion lens. The walls varied in thickness from less than a micron to over 20 micra. Lacunae from the size of two united cell cavities to the size of fifty or sixty cavities were found, but fewer collapsed cells bordered the larger lacunae than were found in no. 7.

No lacunae were found in the medulla, but otherwise this tissue was in a condition similar to that of the inner portion of the cortex.

SUMMARY

A comparison of corresponding regions of the four plants studied is given below.

Integument

NORMAL	NO. 7	NO. 25	NO. 6
200 micra thick	200 micra thick.	200 micra thick.	240 micra thick.
Cuticle 9 micra thick.	Cuticle 12 micra thick	Cuticle 12 micra thick.	Cuticle 12–14 micra thick.
Outer epidermal walls 11 micra thick.	Outer epidermal walls 6.4 micra thick.	Outer epidermal walls 12 micra thick.	Outer epidermal walls 10 micra thick.
Anterior walls of guard cells thicker than posterior walls.	Anterior walls of guard cells thinner than posterior walls.	Anterior walls of guard cells mostly normal but some thinner than posterior walls.	Anterior walls of guard cells normal in some stomata, thinner than posterior walls in others.
Nuclei of epidermal cells 6.5 micra in diameter.	Nuclei of epidermal cells 3 micra in diameter. Thickened granular zone in peripheral portion of nucleoplasm.	Nuclei of epidermal cells 3–5 micra in diameter. Thickened granular peripheral zone in some nuclei.	Nuclei normal.

Palisade

NORMAL	NO. 7	NO. 25	NO. 6
2.5 mm. thick.	2 mm. thick.	1.2–2 mm. thick.	2 mm. thick.
Cytoplasm of cells in layer adhering to wall.	Cytoplasm of cells restricted to one corner of cell.	Cytoplasm of cells in layer adhering to wall.	Cytoplasm of cells in layer adhering to wall.
Chloroplasts numerous, at sides of cells.	No normal chloroplasts.	Chloroplasts reduced to one-half normal number, around sides of cells.	Chloroplasts reduced in number and congregated around nucleus.
Nuclei lenticular, 32–35 micra long, nuclear membrane thin, vacuoles present. Nuclei at side of cell.	Nuclei circular, kidney- or loaf-shaped, 4–6.4 micra long, thickened peripheral layer present, non-vacuolated. Nuclei in corner of cell.	Nuclei like those of no. 7 in shape, 3–7 micra in diameter, narrow peripheral ring present in some which were non-vacuolated. No nuclei at side of cell.	Nuclei mostly like those of no. 7 in shape, 8–12 micra in diameter, outer ring still present in some. Nuclei at sides of cells.

Outer cortex below palisade

NORMAL	NO. 7	NO. 25	NO. 6
Walls of cells 1 micron to 2 micra thick.	Walls 1.5 to 20 micra thick.	Walls 1.5 to 10 micra thick.	Walls 2 to more than 10 micra thick.
Occasional plastids.	No plastids and often no cytoplasm.	A few shrunken plastids around the nucleus.	No plastids.
Nuclei round, 8–12 micra in diameter.	Nuclei, when present round; 5 micra or less in diameter, with thickened peripheral zone.	Nuclei round, 6–7 micra in diameter.	Nuclei round, 8–13 micra in diameter; peripheral zone in some.

Deep cortex

NORMAL	NO. 7	NO. 25	NO. 6
Cell walls 1–3 micra thick.	Cell wal's 1 to 25 micra thick.	Cell walls 3 to over 20 micra thick.	Cell walls 1 to over 20 micra thick.
Cells with cytoplasm in layer adhering to wall and in a network, wall to wall.	Little or no cytoplasm.	Cells varied in amount of cytoplasm.	Cells varied in amount of cytoplasm.
Nuclei round, 8–12, micra in diameter.	Nuclei 5 micra in diameter.	Nuclei 4.5–8 micra in diameter.	Nuclei mostly normal; a few 16–23 micra in diameter.
	Large lacunar spaces present.	A few small lacunae found.	Small lacunae found.

Irregularities in thickness of cell walls are the rule in the parenchyma of desiccated Echinocactus stems. They appear to be due to two opposite processes: small irregularities to unequal drying of walls; large irregularities to hydrolysis of walls. The former accounts for the irregular thickness of the walls of palisade cells and the latter for the gross irregularities in the walls of the cortex. The modification in the thickness of the walls of guard cells must seriously affect the activity of stomata. Where anterior walls of guard cells are much thinner than posterior walls it would appear that increased osmotic pressure in the guard cells should cause a closing or partial closing, instead of an opening of the stoma. Eventually, after the con-

tinued loss of water by the plant, the guard cells would lose turgidity and slowly collapse, and the stomata would remain permanently open.

Recovery from desiccation is rather slow and is manifest first in the peripheral tissues of the plant; in fact, the deeper parts of the cortex never fully recover from the effects of long-continued drying. In parenchyma cells that have remained alive, the cytoplasm increases in amount, vacuoles appear, and nuclei enlarge and may resume normal form. The granular material that had accumulated in the peripheral portion of the nucleoplasm migrates to the central region of the nucleus. Plastids appear in the region of the nucleus in the palisade cells, and with their formation the plant assumes its normal activity. Incidentally the ability to survive such severe treatment as that to which these Echinocactus plants were subjected clearly demonstrates their admirable adaptation to the arid conditions of their native habitat.

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DESCRIPTION OF PLATES

PLATE I

FIG. 1. Transverse section through outer region of stem of Echinocactus no. 7, from a ridge half way between areoles. *a*, integument; *b*, palisade; *c*, spongy tissue of outer cortex. Oval and circular areas are parts of cell walls coming in contact with adjacent cells. (× 46.)

FIG. 2. Transverse section of cuticle and epidermis of Echinocactus no. 7, showing divided ectoderm cell. The lower cell becomes phellogenistic. (× 572.)

FIG. 3. Normal palisade cell, showing position of nucleus and chloroplasts. (× 1200.)

FIG. 4. Transverse section of lacunar region of the cortex of Echinocactus no. 7. Lacunae are shaded in black. (× 1.)

PLATE II

FIG. 1. Nucleus from normal palisade cell of Echinocactus. Note thin membrane, large nucleolus and vacuoles. (× 1165.)

FIG. 2. Nuclei from palisade of Echinocactus no. 7 showing granular zone. Compare with figure 1. (× 1165.)

FIG. 3. Cells from the cortex of normal Echinocactus, just under the palisade tissue, showing small areas of cell walls in contact and appearance of cell contents. Compare with figure 4. (× 317.)

FIG. 4. Cell from the cortex of Echinocactus no. 7, just under palisade tissue, showing somewhat irregular thickness of walls, restricted area of walls in contact, and reduced protoplast. (× 317.)

FIG. 5. Cell from the deep cortex of Echinocactus No. 25, next to phloem of the stele, showing starch content. (× 273.)

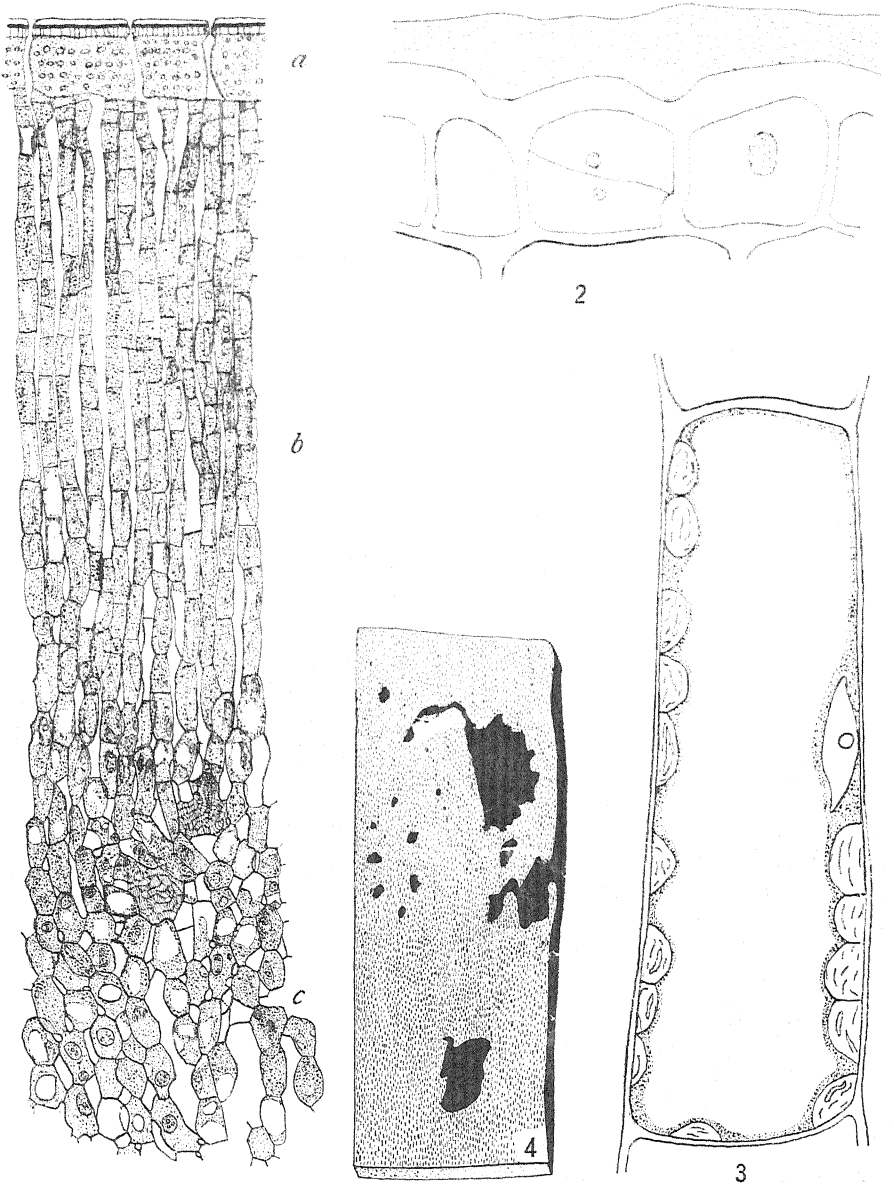
PLATE III

FIG. 1. Palisade cell from Echinocactus no. 7, showing irregular thickness of wall, greatly reduced protoplast, and nucleus with thickened peripheral layer. (× 520.)

FIG. 2. Cortical cell from Echinocactus no. 25, located just outside of phloem, showing irregularities in wall caused by hydrolyzation, and nucleus with granular layer. (× 253.)

FIG. 3. Development of a lacuna in Echinocactus no. 7. *x*, outline of connecting prolongations of two cells: the wall has here almost disappeared. (× 69.)

FIG. 4. Later stage in development of a lacuna in Echinocactus no. 7. A cell containing a disintegrating nucleus is shown at *a*; a mass of gelatinous material resulting from breaking down of several cell walls at *b*. (× 170.)



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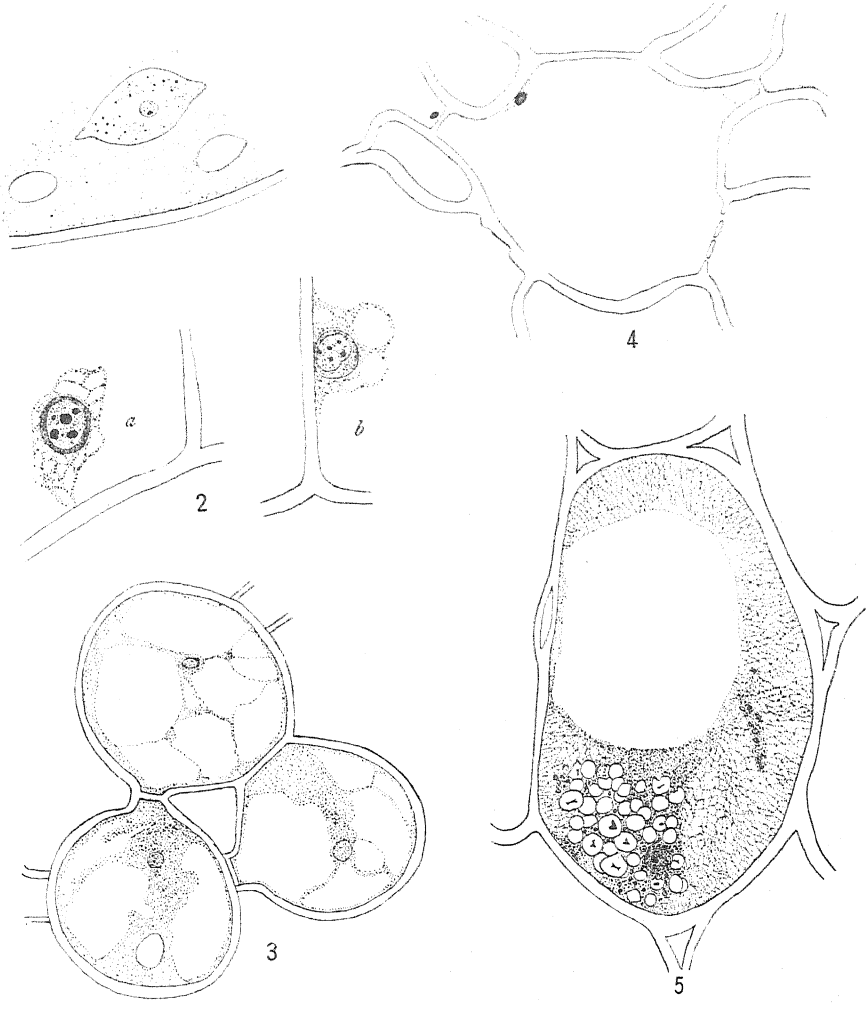

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